Original Contribution

Cytogenetics of Familial Waldenström's Macroglobulinemia: In Pursuit of an Understanding of Genetic Predisposition

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Abstract

Despite recent identification of a recurrent chromosome 6q21 deletion in sporadic Waldenström's macroglobulinemia (WM), elucidation of the molecular pathogenesis of WM remains challenging. In contrast to the growing body of cytogenetic studies in sporadic WM, there have been virtually no informative studies of familial WM. The authors therefore undertook conventional and molecular cytogenetic evaluation of 18 patients with familial WM and 3 patients with immunoglobulin (Ig) M monoclonal gammopathy (IgM-MG) from 15 families to determine the nature and extent of chromosomal abnormalities associated with familial WM. The frequency and distribution of chromosomal changes in familial WM resembled those in sporadic WM, including lack of *IgH* rearrangements and t(9;14); however, we detected del6q21 in only 1 patient. Occasional findings appeared to be novel; however, none were recurrent, and their significance remains unclear. Only one abnormality found in bone marrow specimens was detected in parallel peripheral blood lymphocyte studies, suggesting that most abnormalities represented somatic changes. Although they must be viewed in light of the hypoproliferative nature of WM, our results suggest that further progress in delineating the genetic determinants of WM susceptibility might be gained from alternative approaches such as candidate gene or linkage analysis.

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Introduction

Elucidation of the molecular basis of Waldenström's macroglobulinemia (WM) has been elusive. Despite cytogenetic reports since the 1960s, few recurrent cytogenetic abnormalities have been described. This contrasts with most other lymphoproliferative disorders and has been attributed to the hypoproliferative nature of WM. However, recently a specific deletion of the long arm of chromosome 6, del6q21,[1] has been found in ≤ 50% of patients with sporadic WM who have any cytogenetic abnormality shown by conventional G-banding[2] or more sensitive molecular cytogenetics methods.[3]

Although rare case reports exist,[4-6] familial WM has not been studied with current cytogenetic methods. Such family studies might contribute important clues regarding the genetic basis for disease susceptibility. The authors therefore investigated a series of patients with familial WM, employing conventional G-banding and molecular cytogenetic methods, to determine the nature and extent of chromosomal abnormalities associated with familial WM. We also included first-degree relatives with asymptomatic immunoglobulin (Ig) M monoclonal gammopathy (IgM-MG)[7] because of previous cytogenetic findings in patients with monoclonal gammopathy of uncertain significance (MGUS).[8]

Patients and Methods

Patients

Families with ≥ 2 cases of WM, or WM in combination with related lymphoproliferative disorders, were eligible for study. We clinically evaluated all available affected individuals and their first-degree relatives, obtaining pathology reports on all cases when possible to confirm diagnoses. All patients, and any first-degree relatives with IgM-MG on immunofixation electrophoresis, were asked to provide bone marrow and peripheral blood samples for cytogenetic evaluation. Treatment status did not affect eligibility. Twenty-one patients fulfilled these criteria (Table 1). This study was conducted under institutional review board approval, and all patients gave informed consent for sample collection and analysis.

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Standard Cytogenetics, Spectral Karyotyping, and Interphase Fluorescence In Situ Hybridization

All cytogenetic analyses were conducted under contract to Cancer Genetics, Inc. Bone marrow specimens were processed in parallel 24-hour unstimulated and 72-hour mitogen-stimulated cultures, as previously described.[9] Peripheral blood specimens were processed as 72-hour mitogen-stimulated cultures. At least 20 banded metaphases were analyzed for each patient whenever possible, and ≥ 2 karyotypes were prepared from each clone. Each karyotype was described according to the International System for Human Cytogenetic Nomenclature (1995).

Additional slides from each culture were prepared for spectral karyotyping (SKY) studies, which were performed on freshly prepared slides as previously described[10] using the SKYPaint® Probe, and imaged using the SD200 SpectraCube spectral imaging system.

Interphase fluorescence in situ hybridization (FISH) was performed on freshly prepared direct preparations of bone marrow cells, as described.[11] We screened for rearrangements at the IgH locus using an IgH probe. To screen for t(11;14)(q13;q32), del13q14.3, and chromosome 11 aneuploidy, we used the LSI IGH and CCND1 dual color/dual fusion detection probe, the D13S25 single-color probe, and the CEP11 centromere enumeration probe, respectively. All probes were validated before use, and commercial probes were prepared and applied according to the manufacturer's direction. A minimum of 500 nuclei were scored for each set of probes whenever possible.

Comparative Genomic Hybridization

DNA extracted from bone marrow apirates was examined for copy number changes as described.[12] At least 8 chromosome metaphases were imaged and analyzed with the Quips XL image analysis program. The comparative genomic hybridization (CGH) red:green ratio profiles of 0.80 and 1.20 suggested loss and gain of DNA sequences, respectively.

Table 1 Patient Characteristics at the Time of Cytogenetic Evaluation

Characteristic	Value		
Number of Families	15		
Patients with WM	18		
Sex			
Male	12 (67%)		
Female	6 (33%)		
Age at diagnosis			
Median	57 Years		
Range	37-76 Years		
Age at evaluation			
Median	59.5 Years		
Range	41-81 Years		
Previous chemotherapy			
Yes	7 (39%)		
No*	11 (61%)		
IgM level			
Median	1955 mg/dL		
Range	134-5230 mg/dL		
Patients with IgM-MG	Patient 1	Patient 2	Patient 3
Sex	Male	Male	Female
Age at diagnosis	65 Years	79 Years	66 Years
IgM level	2080 mg/dL	628 mg/dL	766 mg/dL
Bone marrow histology	Normal	Normal	Normal

* Includes 3 patients treated with single-agent interferon-α2b (n = 2) or rituximab (n = 1).

Results

All patients had sufficient metaphases to be evaluable. Nine patients with WM (50%) had no abnormal findings by all methods (Table 2), likely related to low tumor burden and/or outgrowth of normal marrow elements in culture. No patients with IgM-MG, including one having loss of chromosome Y as the sole abnormality, had significant cytogenetic findings. We observed abnormalities in 9 patients (5 untreated) with WM (50%), 5 of which (56%) were identified by conventional G-banding. Five abnormalities were clonal. Numeric aberrations included trisomy 1 and monosomy 11 in 1 patient each. Structural abnormalities included 2 cases each with loss of 7q material or rearrangement involving chromosome 7, respectively, and 1 instance each of rearrangements of chromosomes 1, 11, and 17, deletion of 6q21, and inversions of chromosomes 3 and 19. We found no t(9;14) translocations.

Because G-banding alone can produce false-negative findings,[13] we also performed SKY and/or CGH in all patients. Spectral karyotyping provided additional information in 5 patients. Comparative genomic hybridization contributed independent evidence of interstitial loss of chromosome 1p36 DNA sequences in 1 patient with uninformative G-banding. No patient studied by FISH had *IgH* gene rearrangements. Representative studies are shown in Figure 1. We also conducted a pilot study in 5 patients with specific FISH probes to detect rearrangement and deletion of chromosomes 11q13 and 13q14.3, respectively, which have been associated with MGUS,[8] and for aneuploidy of chromosome 11, which has been described in WM.[14] We did not detect abnormalities by any of these assays.

Discussion

Despite descriptions of WM familial clusters for > 40 years, cytogenetic evaluation of familial WM has been limited and the findings inconsistent.[4-7,15,16] These reports largely predated the modern cytogenetic era. More recently, some familial cases have exhibited intrafamilial sharing of distinct human

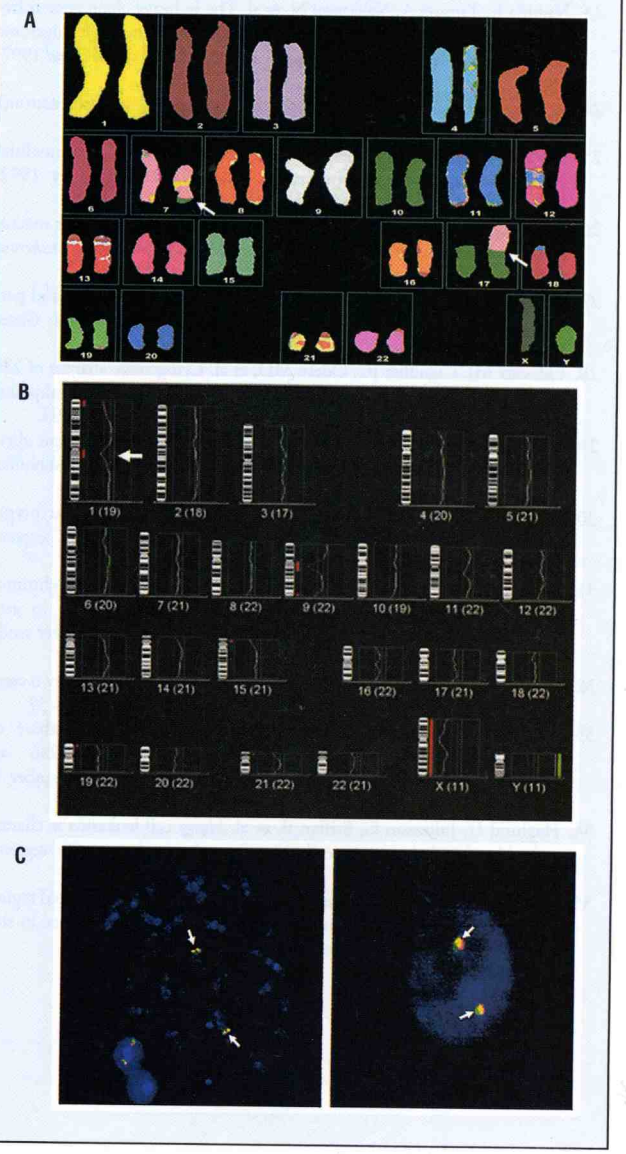
Table 2 Karyotype Results in Patients with Familial Waldenström's Macroglobulinemia or IgM Monoclonal Gammopathy

Patient	Family	IgM	Karyotype	Additional Information Provided	
Patients with WM, Treated				**SKY**	**CGH**
1	A	3400 mg/dL	46,XY[20]	None	Loss, 1p36.1 – 1p36.3
2	B	1960 mg/dL	46,XY[30]	None	None
3	C	468 mg/dL	46,XY,+1,dic(1;7)(p10;q10)[3]/46,XY[9]	46,XY,t(1;7)[2]	None
4	C	1090 mg/dL	46,XX[20]	None	None
5	D	4890 mg/dL	46,XY[20]	46,XY,t(7;17)[1]	None
6	E	1220 mg/dL	46,XY,t(7;11)(p15;p15)[3]/46,XY[20]	None	None
7	F	5120 mg/dL	46,XX[30]	None	None
Patients with WM, Untreated					
8	G	962 mg/dL	46,XY[20]	None	None
9	H	5100 mg/dL	46,XY[20]	None	None
10	A	1630 mg/dL	46,XY,t(7;14)(q32;q32)[1]/46,XY[20]	None	None
11	I	1950 mg/dL	46,XX[20]	None	None
12	I	652 mg/dL	46,XX[24]	None	None
13	D	2120 mg/dL	46,XY[30]	46,XX,+inv(3)(p21q24),-11[3]/ 46,XX,+inv(3)(p21q24)x2,-11,-11[1]/ 45,XX,+del(15q),-18,-22[1]/46,XX[15]	None
14	J	5230 mg/dL	46,XY[30]	None	None
15	K	134 mg/dL	46,XX[30]	45,XX,-18[1]	None
16	L	1100 mg/dL	46,XY[30]	None	None
17	L	2000 mg/dL	46,XY,inv(19)(p13.3q13.1)[30]	46,XY,inv(19)(p13.3q13.1), der(2)t(2;9)(q21-31?; q21),del(9)q[1]/ 44,XY,inv(19)(p13.3q13.1),-5 -13[1]	None
18	M	3950 mg/dL	46,XY,del(6)(q21),del(7)(q31q36)[2]/ 46,XY, del(6)(q21),del(7)(q31q36), t(17;17)(p11.2;q25)[5]/46,XY[26]	46,XY,der(1)t(1;6)(p?;q?)[1]	None
Patients with IgM-MG					
19	N	766 mg/dL	46,XX[20]	None	None
20	D	2080 mg/dL	46,XY[20]	None	None
21	O	628 mg/dL	45,X,-Y[19]/46,XY[11]	None	None

leukocyte antigen (HLA) haplotypes,[17-19] suggesting possible linkage of WM susceptibility to HLA-associated genes.

This study represents the first systematic cytogenetic analysis of a series of well-characterized familial WM and IgM-MG cases. Our results are generally consistent with those seen in sporadic WM.[1,2,14,20-31] Cytogenetic abnormalities were detected in approximately 50% of patients, and no cases were found to have the clonal 14q32 rearrangements involving the *IgH* gene or the t(9;14) associated with a subset of lymphoplasmacytic lymphomas.[3] There were 3 apparently novel findings. One previously treated patient had a dic(1;7)(p10;q10), resulting in trisomy for most of chromosome 1 and monosomy for chromosome 7q. Balanced t(1;7) translocations are associated with myelodysplasia and acute myeloblastic leukemia, and may reflect prior chemotherapy.[32] Two untreated patients had an inversion of chromosome 3 or 19, respectively, with the former occurring as part of a complex karyotype and the latter as the sole clonal abnormality. Inversions may be normal constitutional findings; however, we found only the inv19 in the patient's peripheral blood lymphocytes. Inversions might also indicate submicroscopic deletions; however, CGH revealed no loss of DNA in these patients. Paracentric inversions

Figure 1 Spectral Karyotyping, Comparative Genomic Hybridization, and Fluorescence In Situ Hybridization Showing Conventional G-Banding



Representative data provided by SKY, CGH, and FISH in cases found to be uninformative by conventional G-banding. (**A**) Spectral karyotype shows a rearrangement involving the long arms of chromosomes 7 and 17 (arrows). (**B**) Comparative genomic hybridization reveals loss of DNA sequences at chromosome 1p36.1-1p36.3 (region depicted in red, arrow). (**C**) Interphase FISH using a dual-color/dual-fusion probe for the *IgH* locus on chromosome 14q32. Only 2 fusion signals appear in the single metaphase spread or in the interphase nucleus (arrows), indicating no rearrangement at the *IgH* locus.

of chromosome 3q are associated with acute myeloblastic leukemia, and pericentric inversions have been described in a single lymphoproliferative tumor, a diffuse large-cell lymphoma.[33] In contrast, inv19(p13q13) is described as a recurrent abnormality in hairy cell leukemia.[34] The significance of these findings in familial WM remains undefined.

In contrast to recent reports of 6q21 deletions in as many as half of sporadic WM cases,[2,3] we found del6q21 as part of a complex karyotype in only 1 patient with familial WM. Comparative genomic hybridization failed to demonstrate this deletion in any other patient. The deletion was not likely of germline origin, given its absence in peripheral blood lymphocytes, and we were unable to assess intrafamilial concordance. The region encompassing 6q21 is under intense investigation; it harbors reasonable candidate tumor suppressor genes,[35] the loss of which may predispose a patient to development of WM. It remains unclear whether the del6q21 is associated with disease initiation or progression. Although a gene(s) in this region may contribute to a subset of familial WM, given our results it is unlikely to represent a universal initiating event in familial WM susceptibility.

Any germline chromosomal changes associated with familial WM might be expected to be present among relatives with IgM-MG, despite the low prevalence of clonal cells in their bone marrow. Although based on very small numbers, we found no abnormalities in any of the patients with IgM-MG in this sample.

Our results must be viewed in the context of the hypoproliferative nature of WM and low tumor burden resulting from prior therapy or early diagnosis. Immunomagnetic selection to enrich the sample for the malignant clone might have increased the detection rate in this series;[3] however, the overall prevalence of abnormalities observed in this study is similar to that reported with use of selection techniques. Because of the late age distribution and mortality of WM, we seldom had access to other affected family members for study and were thus often unable to assess familial concordance for specific cytogenetic abnormalities. However, with the exception of inv19, none appeared to be germline, based on their exclusive presence in tumor. Nevertheless, very small deletions may have been missed.

Conclusion

In summary, cytogenetic evaluation of this cohort of familial WM revealed no increased frequency of chromosomal abnormalities compared with sporadic cases. As expected, sensitive techniques such as SKY and CGH provided additional information beyond that yielded by G-banding alone. However, none of the observed abnormalities, including novel findings, appear to be characteristic of familial WM, given their somatic tissue distribution and lack of intra- and interfamilial concordance. Thus, it is likely that successful identification of susceptibility gene(s) in familial WM will require alternative approaches, such as candidate gene or linkage analysis.

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